

Fig. 1A Fig. 1C Fig. 1E

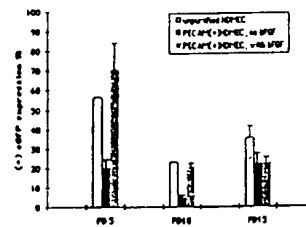
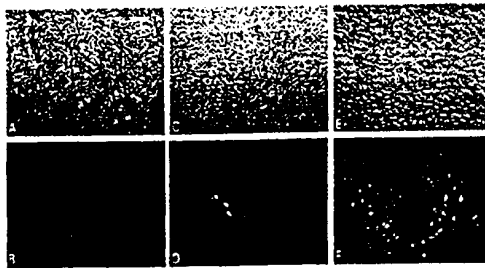


Fig. 1B Fig. 1D Fig. 1F

Fig. 2



Fig. 3A

Fig. 3B

Fig. 4A

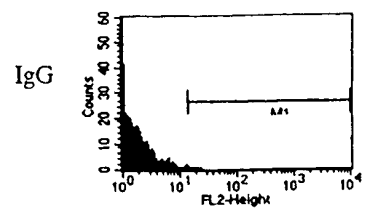


Fig. 4B

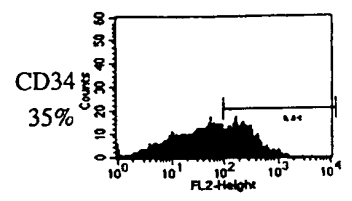
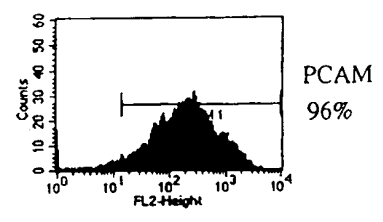


Fig. 4C

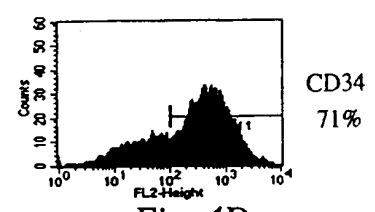


Fig. 4D

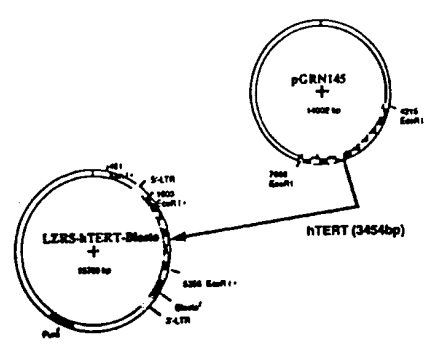


Fig. 5

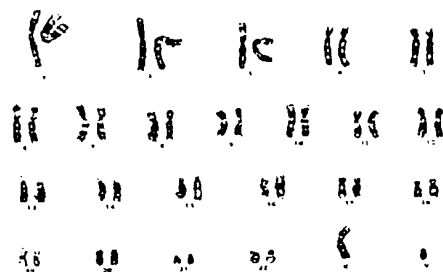


Fig. 8

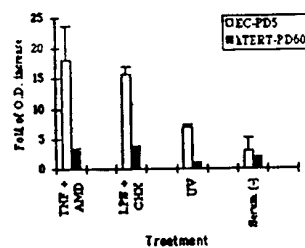


Fig. 9A

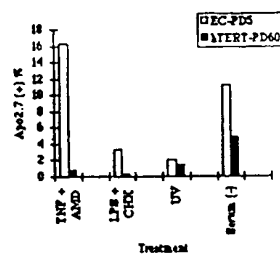


Fig. 9B

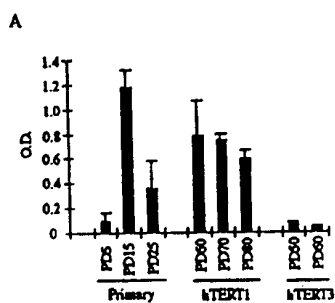


Fig. 10A

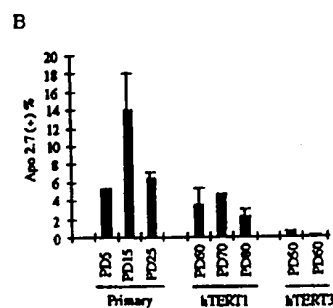


Fig. 10B

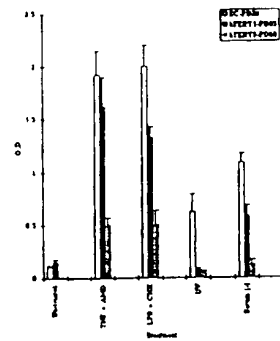


Fig. 11A

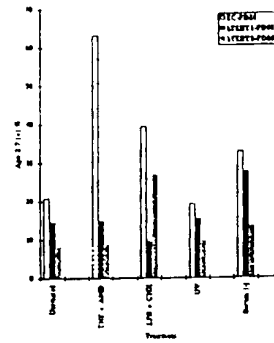


Fig. 11B

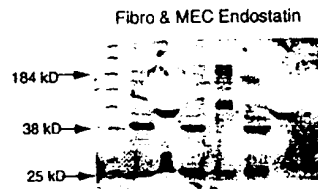


Fig. 12

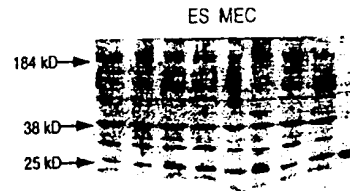


Fig. 13

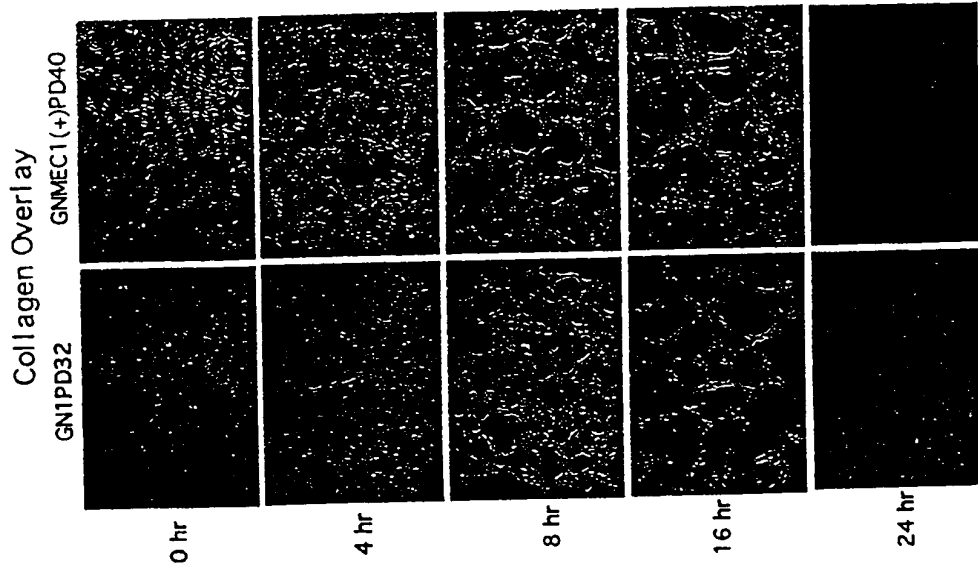


Figure 14

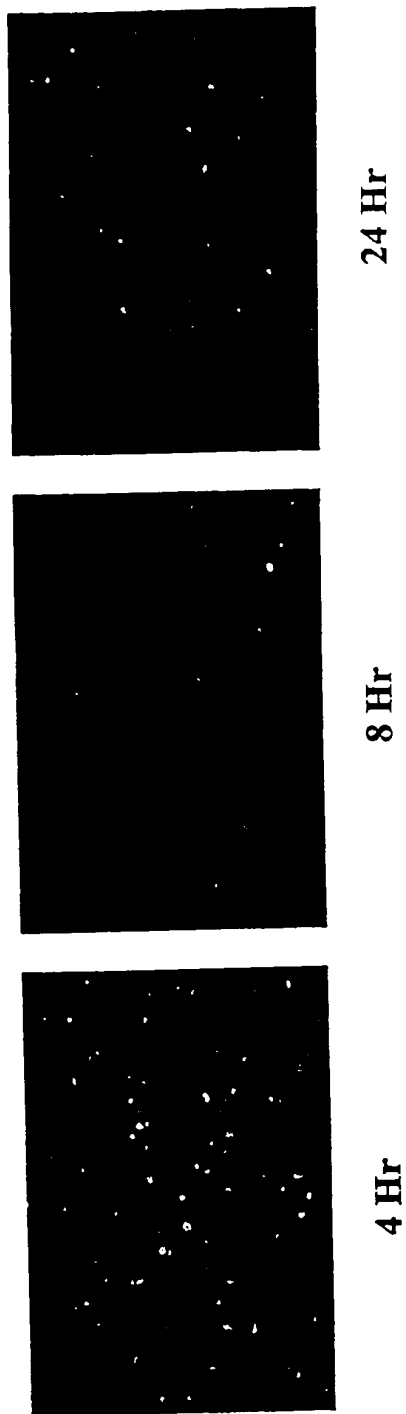
Time course of apoptotic induction by 3D collagen overlay *in vitro* and superior survival of Tert-EC versus primary parental EC. By 16hr after collagen overlay, parental cultures are undergoing dissolution and apoptosis whereas, Tert-EC are still forming tubule structures. At 24hr, all parental cells are dead and Tert-EC are stable.

GN1PD32: eGFP-labeled primary HDMEC at PD32 (mid-late passage)

GNMEC1(+)/PD40: hTERT(+)
 GNMEC-1 at PD40

Figure 15

Time Course of High Density Matrigel Tubule Formation



Demonstration of the utility of using eGFP-labeled Tert-EC for tracking morphogenetic patterns of cells forming microvascular structures *in vitro*

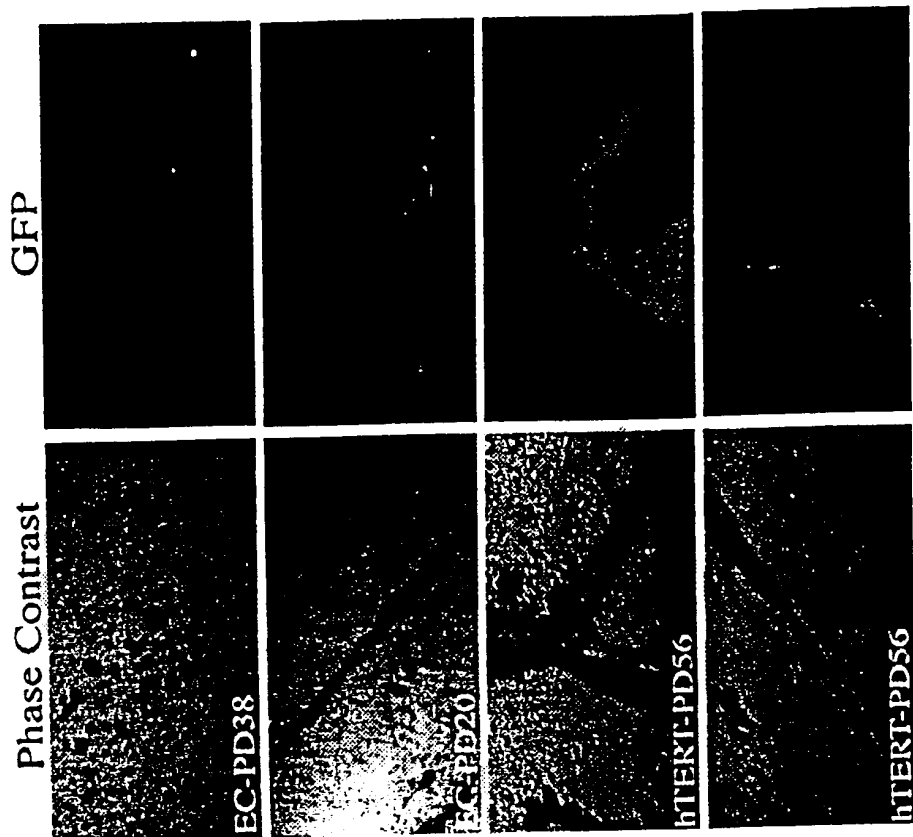


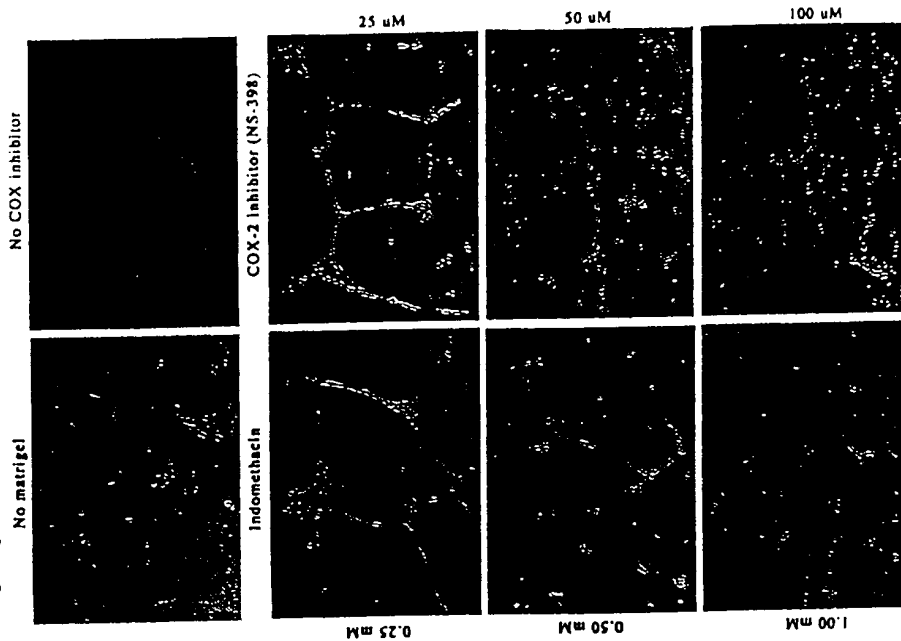
Figure 16

Superiority and utility of eGFP-labeled Tert-EC in formation of vascular structures in 3D Matrigel *in vitro*.

Senescent parental EC (EC-PD38) show no vessels in 3D Matrigel compared to younger cells (EC-PD20). Vessels were numerous and prominently labeled in all Tert-EC cultures (hTERT1-PD56).

Figure 17

Inhibition of *in vitro* angiogenic web formation by cyclo-oxygenase (COX) antagonists



Tert-EC were incubated in the presence of two different COX inhibitors (indomethacin and NS-398) at different concentrations and examined 12hr after plating on Matrigel. Controls show no vessels in absence of Matrigel and numerous vessels in absence of or at low dose COX blockers. Graded inhibition of vessel formation is observed with increasing doses of COX blockers, with the COX-2 specific inhibitor (NS-398) 10-fold more potent than the general inhibitor, indomethacin.

SCID-Mouse Matrigel Implantation of Human HDMEC

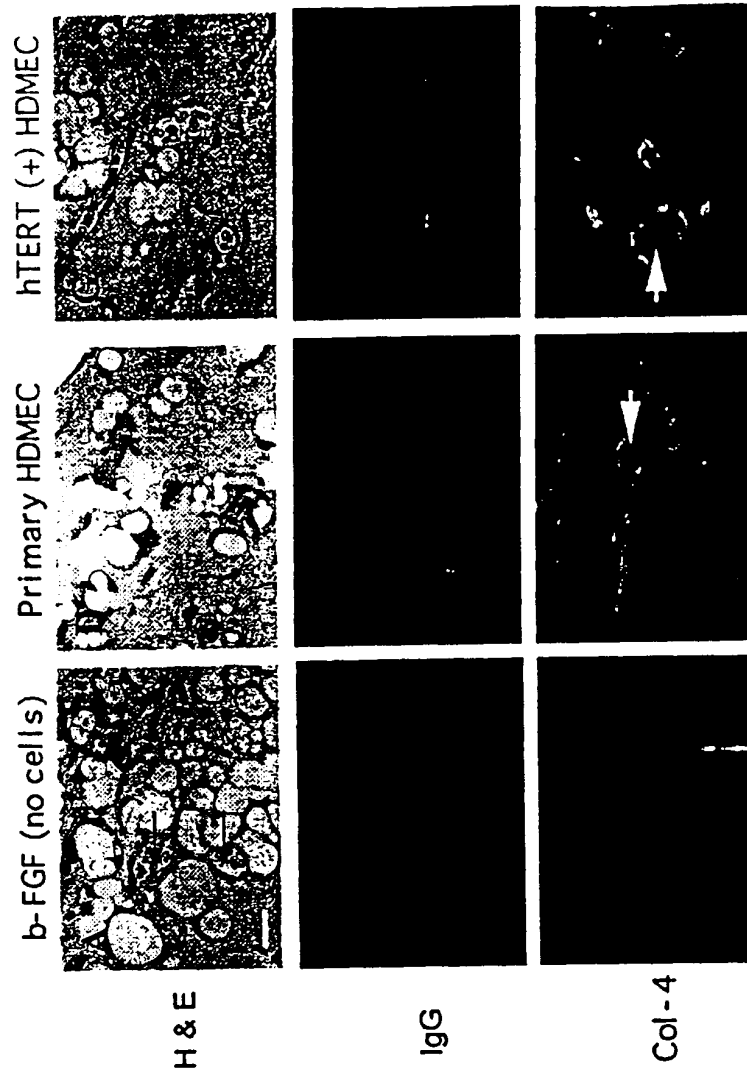


Figure 18

Tissue sections stained with H&E (A,D,G) and for anti-human Type 4 collagen (C,F,I) demonstrate the presence of host murine vessels in Matrigel implants containing bFGF (arrows) but absence of human basement membrane collagen (C). Both primary and Tert-EC demonstrate Col-4(+) vessels containing RBCs (arrows). IgG controls shows no IF reactivity (B,E,H). Results indicate human vessels have formed anastomoses with host mouse vessels (i.e. "chimeric" vessels).

Figure 19 Superiority of Tert-EC at In Vivo Microvessel Formation

Early-mid passage parental eGFP-labeled HDMEC (GN1-PD20) were directly compared to Tert-EC line (GNMEC-1) for their ability to form fluorescent microvessels *in vivo* using the SCID mouse Matrigel implantation assay system. TertEC demonstrated more fluorescent vascular structures at all time points after implantation versus parental line.

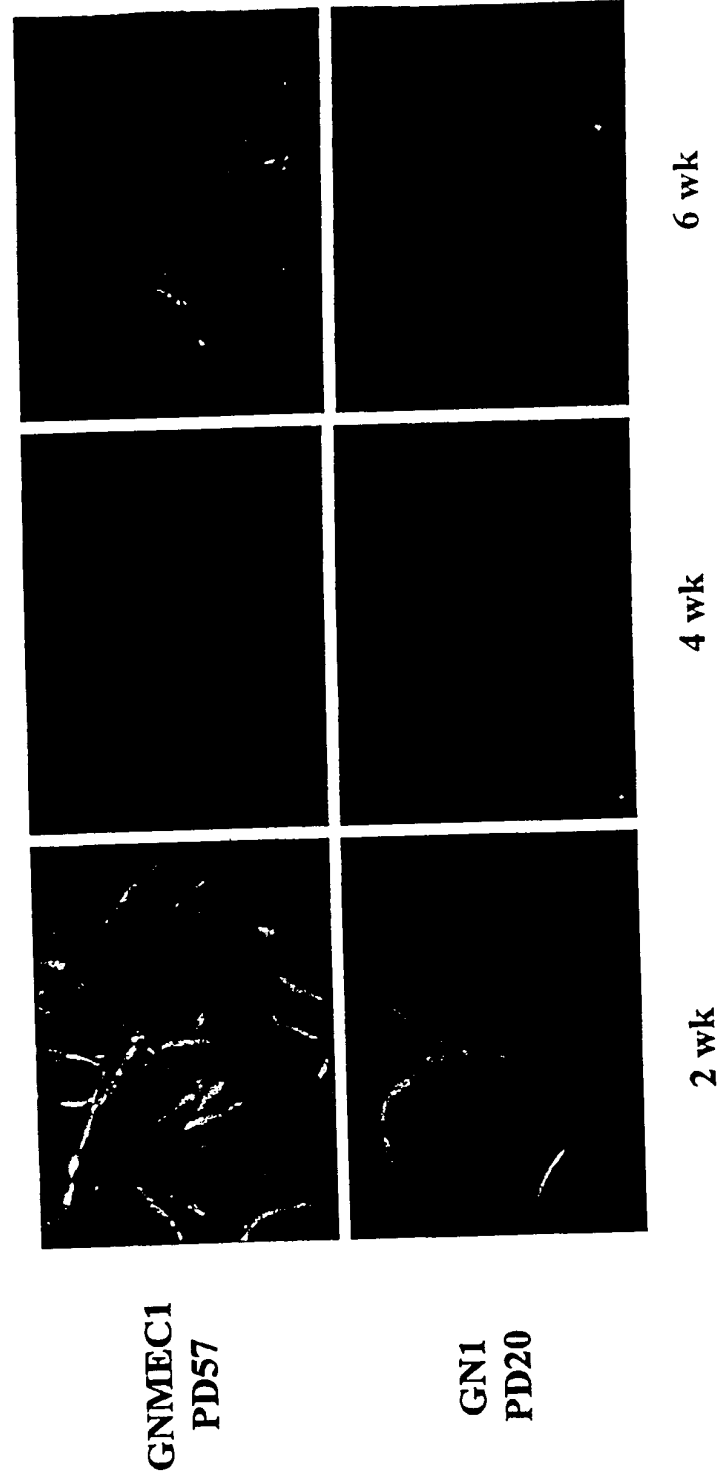


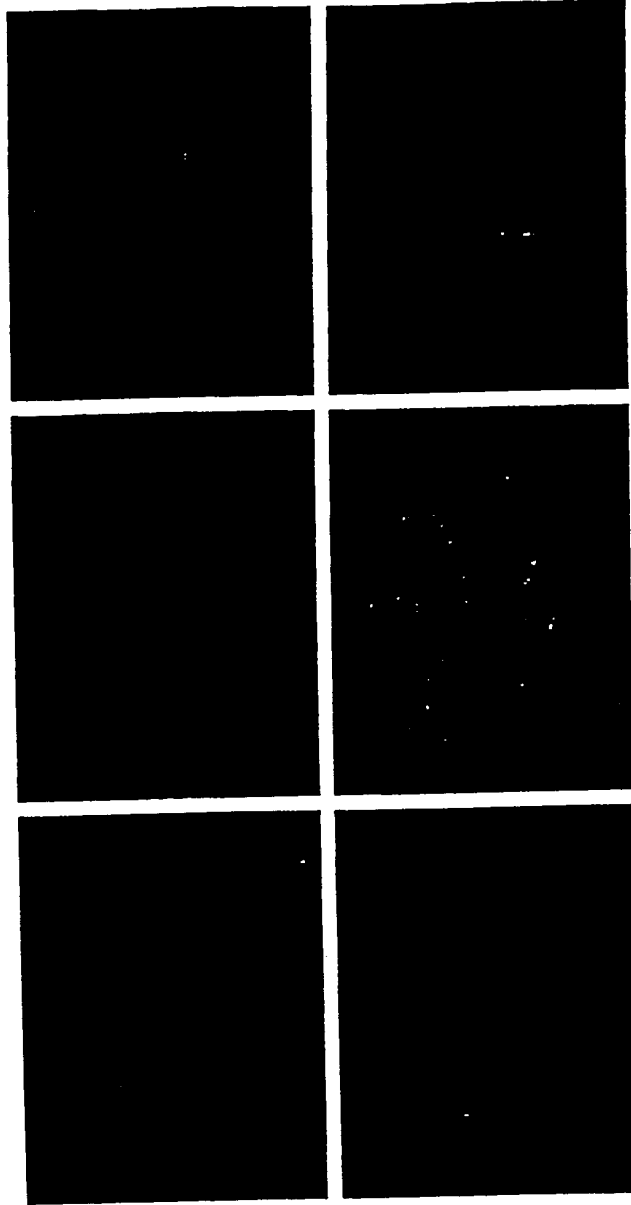
Figure 20 Superiority of Tert-EC at In Vivo Microvessel Formation

Use of Type 4 collagen IF reactivity to quantify human microvessels *in vivo* demonstrates superior durability of Tert-EC (GNMEC1) versus late passage parental cells (GN1PD34). Younger parental cells (PD20) show many small diameter vessels.

GNMEC1PD57

GN1PD20

GN1PD34



IgG

Col 4

Figure 21

Superiority of Tert-EC at In Vivo Microvessel Formation

Quantitative comparison of microvessel density by IF micromorphometry using anti-human Type 4 collagen demonstrates equal number of vessels in young primary (GN1PD12) vs Tert-EC (GNMEC1) and maintenance of vessels at 2, 4 and 6 weeks after implantation in Tert-EC (>25); whereas, mid and late passage parental EC show both decreased numbers and loss of vessels with time *in vivo*. Dermal fibroblasts (Fb) and human fibrosarcoma cells (HT-1080) show no vessel formation (see Figures 22, 23).

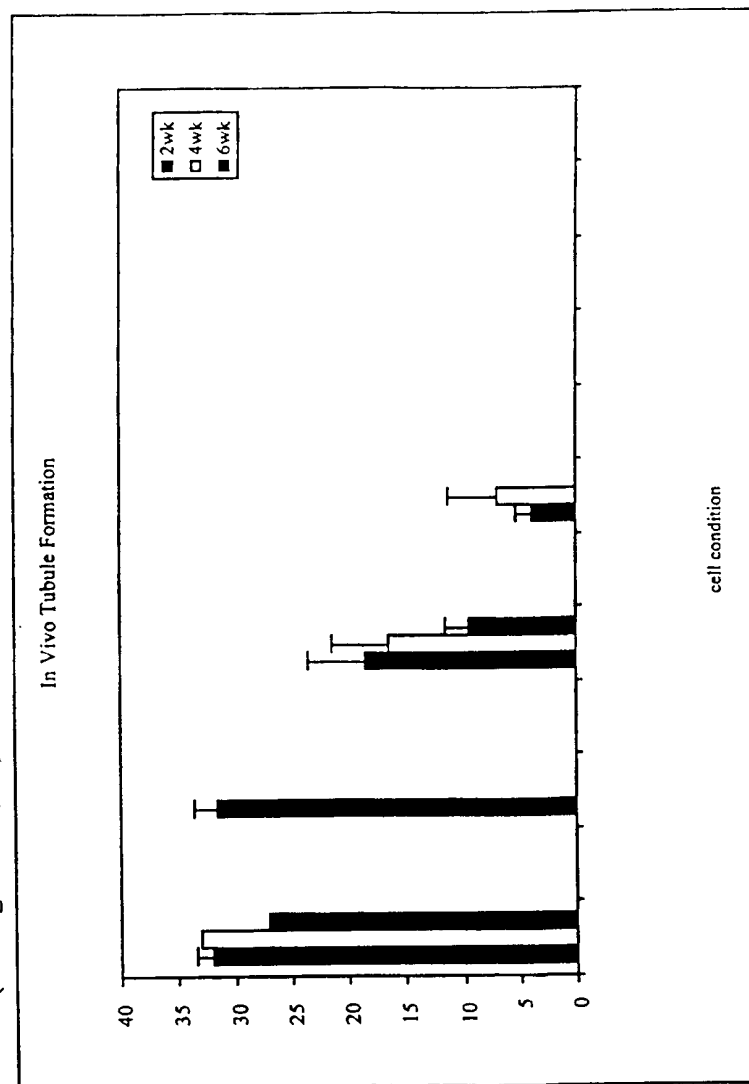
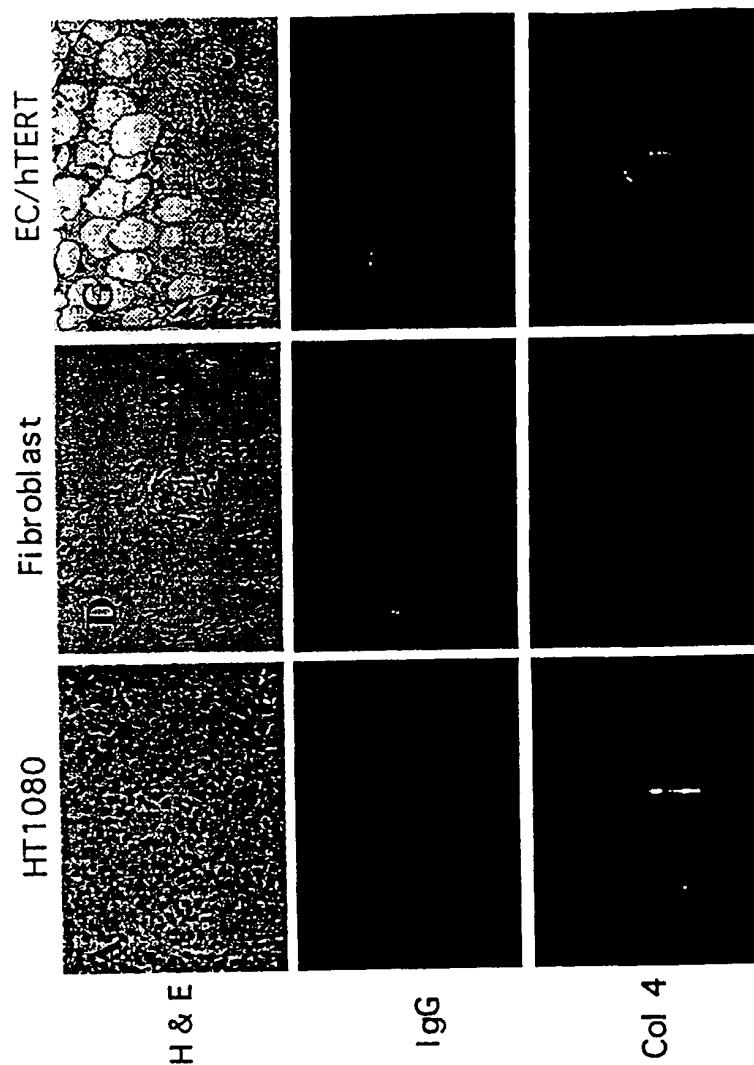


Figure 22

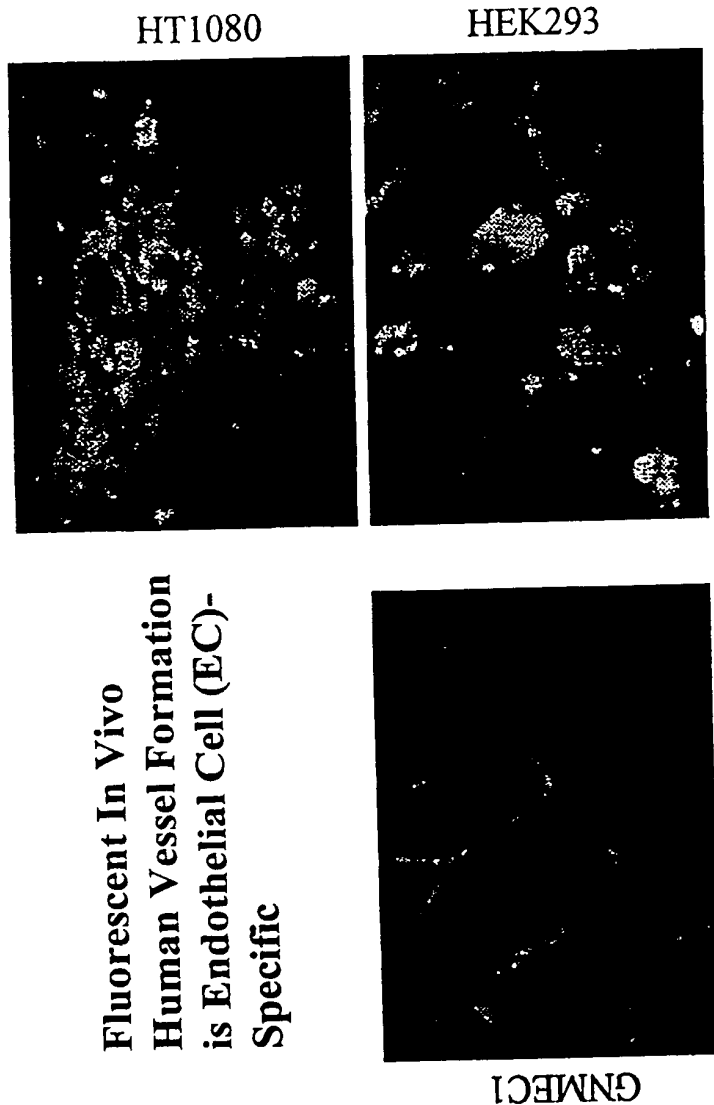
Specificity of Tert-EC at In Vivo Microvessel Formation



Tissue sections stained with H&E (A,D,G) and for anti-human Type 4 collagen (C,F,I) demonstrate the presence of human vessels in Matrigel implants containing Tert-EC (G,I) but absence of human basement membrane collagen (C,F) in implants containing HT1080 cells or human dermal fibroblasts. IgG controls shows no IF reactivity (B,E,H). Results indicate lack of human vessel formation in implants containing nonEC types

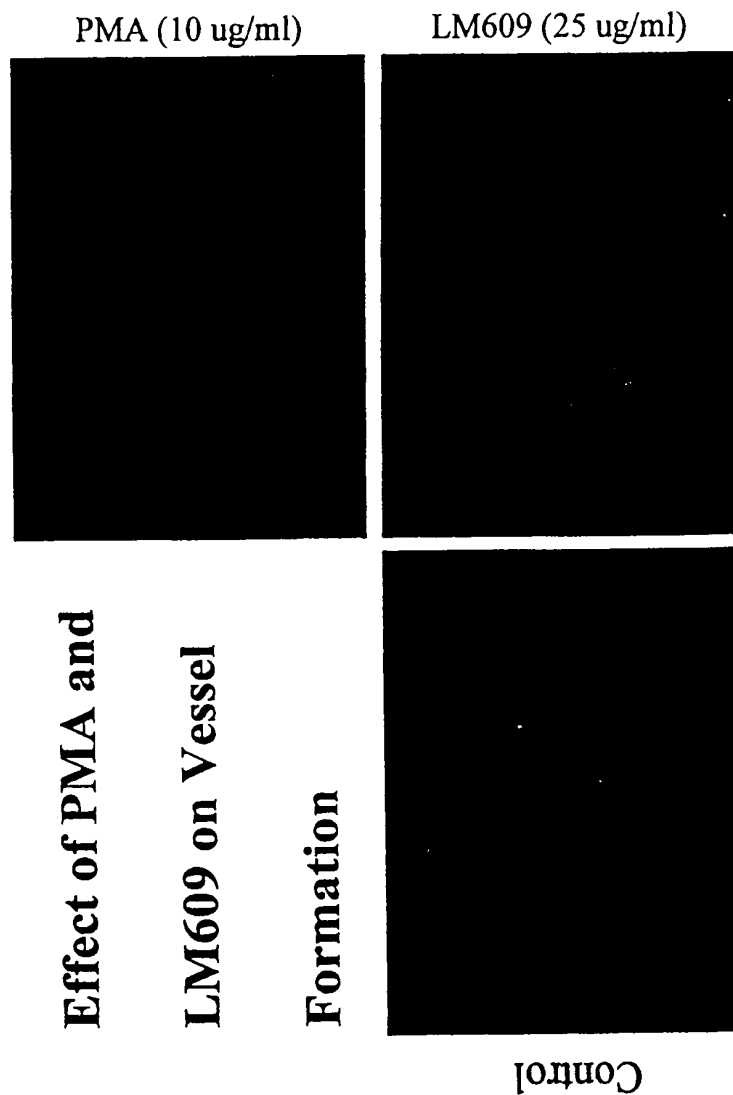
Figure 23

**Fluorescent In Vivo
 Human Vessel Formation
 is Endothelial Cell (EC)-
 Specific**



Human fibrosarcoma cells (HT1080) and human embryonic kidney tumor cells (HEK293) expressing eGFP show fluorescent tumor masses but no microvessels, whereas, Tert-EC (GNMEC1) form obvious fluorescent vessels.

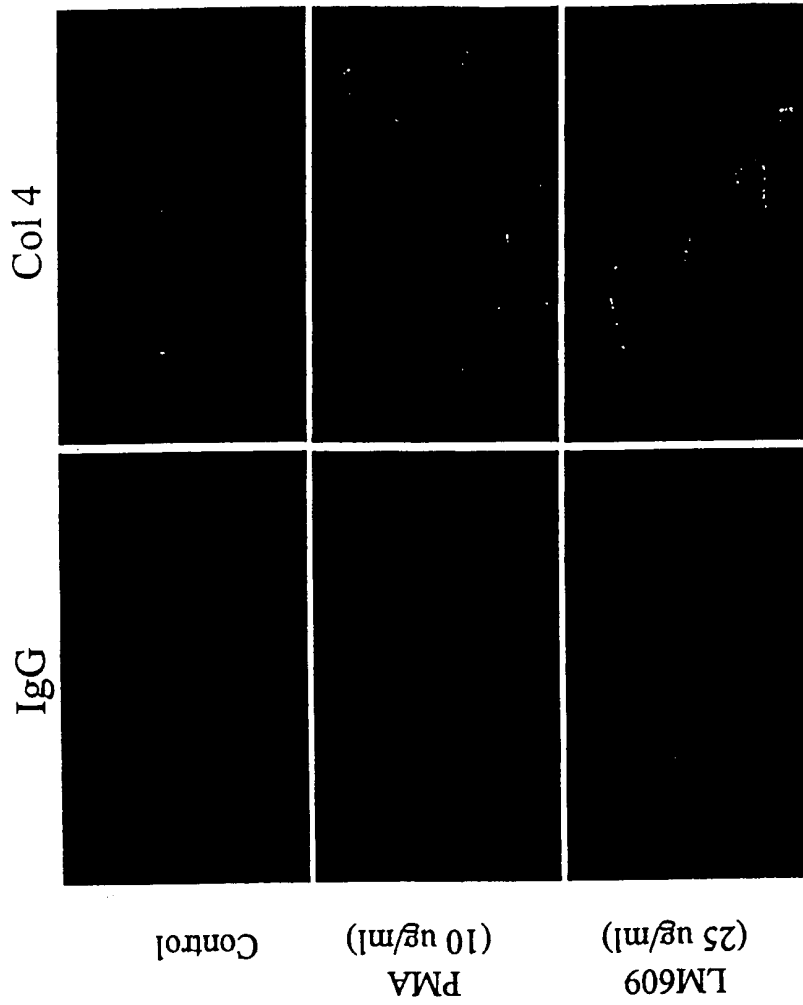
Figure 24



Treatment of Tert-EC with phorbol ester (PMA) or vitronectin receptor ($\alpha_v\beta_3$) -antagonist (LM609) for 2 hrs prior to implantation *in vivo*. Results show PMA has a slight negative effect on vessel density, whereas, LM609 appears to increase vessels relative to control, untreated cells.

Figure 25

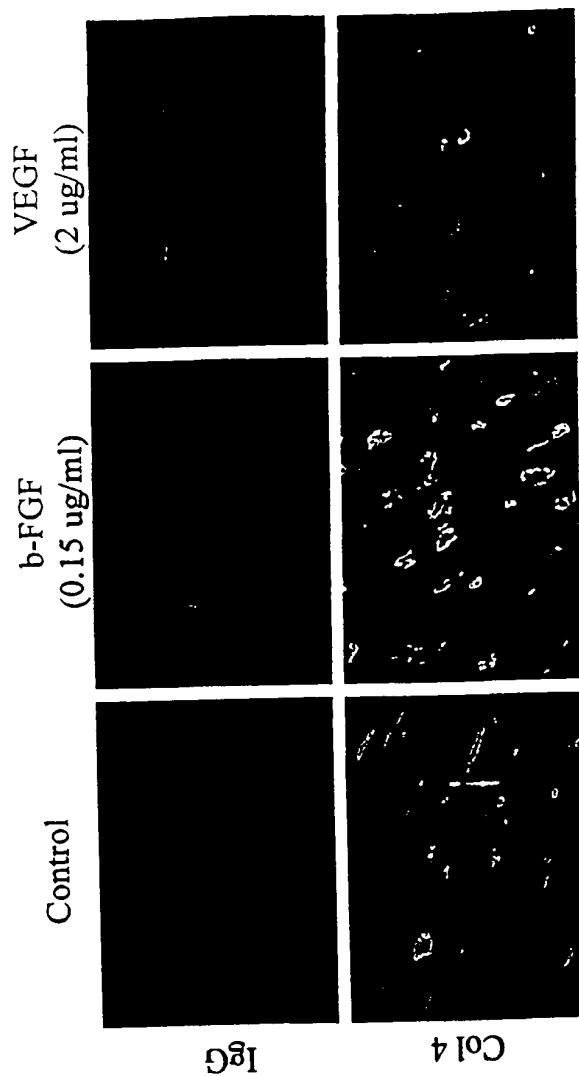
Effect of PMA and LM609 on *In Vivo* Vessel Formation



Quantification of vessel density by IF staining of human Type 4 collagen shows effects of PMA and LM609, as described in Figure 24.

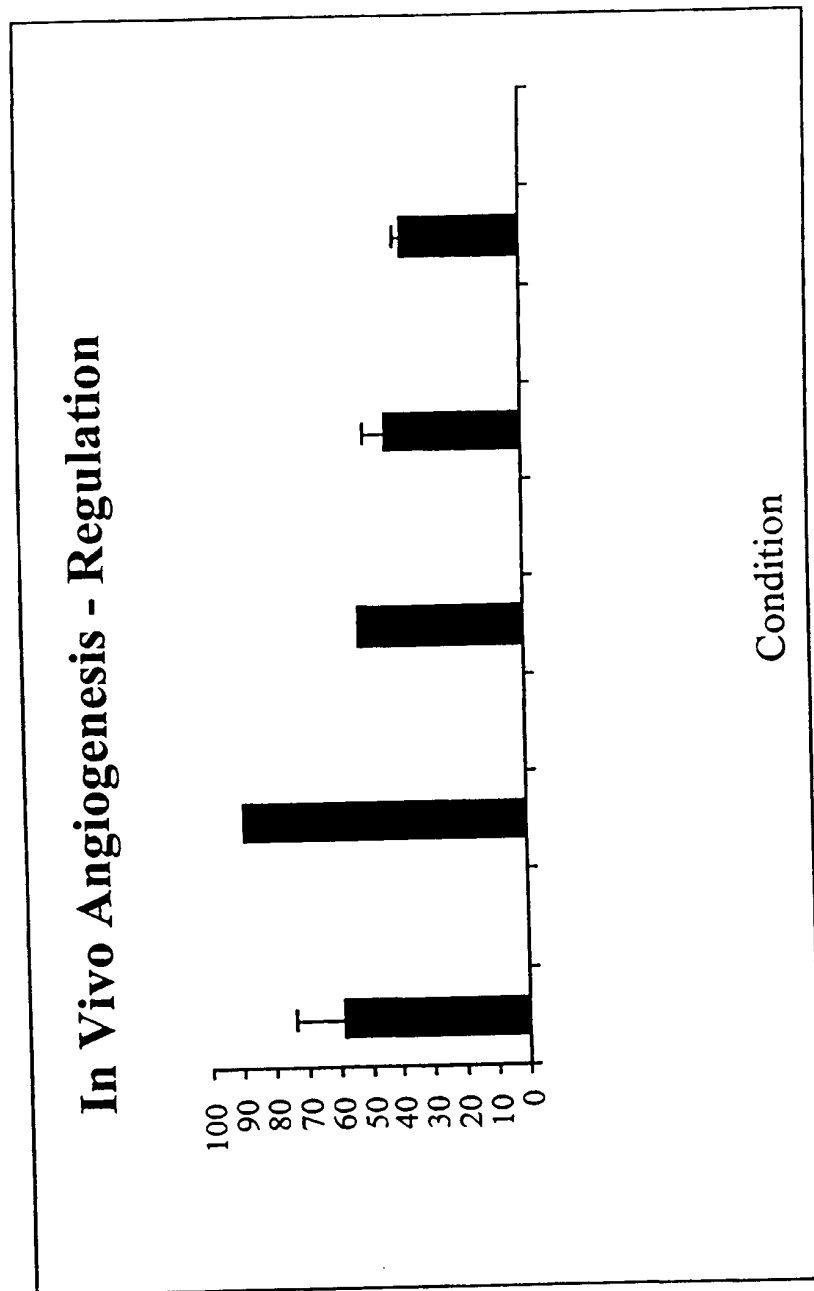
Figure 26

Effect of Growth Factors on Vessel Formation



Quantification of vessel density by IF staining of human Type 4 collagen shows effects of b-FGF and VEGF. Results indicate FGF increases vessel density, whereas, VEGF has little effect.

Figure 27



Effect of different angiogenic regulators on GNMEC1 *in vivo* microvessel formation. Results show significant changes in vessel density only by b-FGF.

FIGURE 28

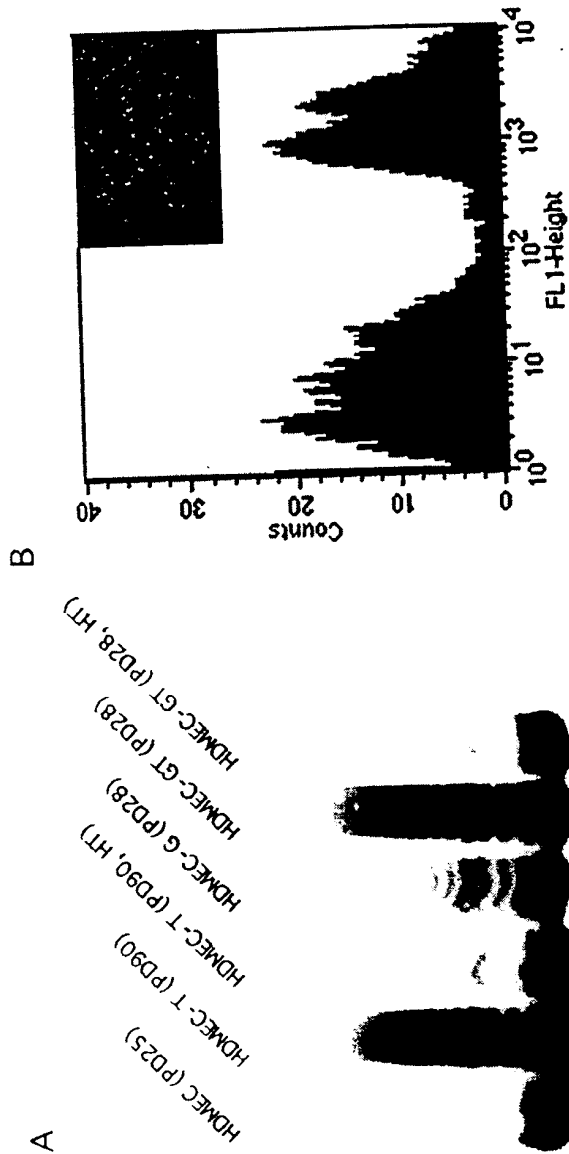


FIGURE 29

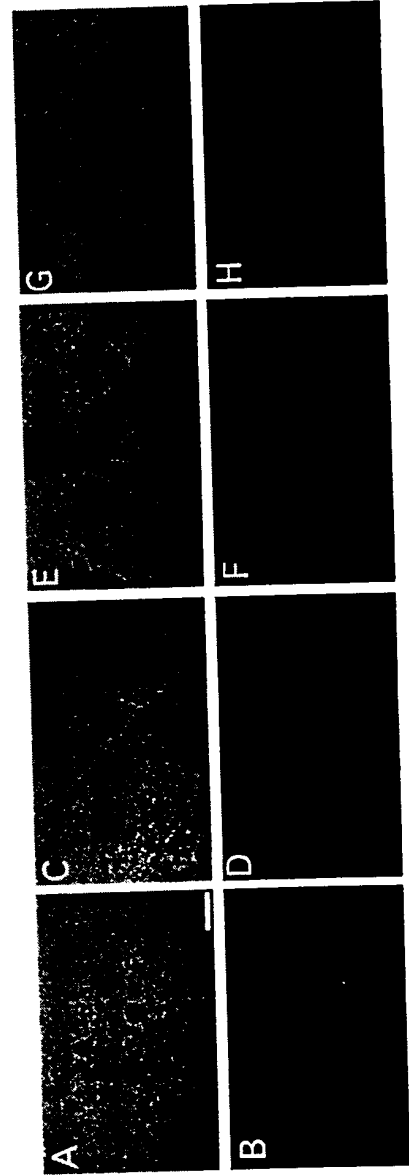


FIGURE 30

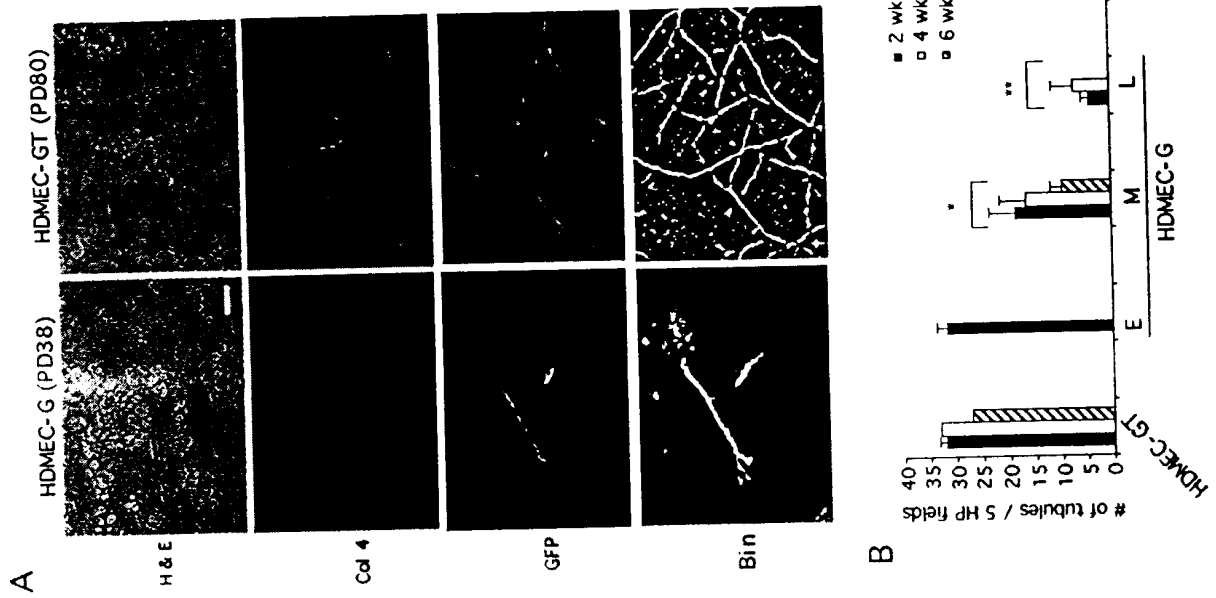


FIGURE 31

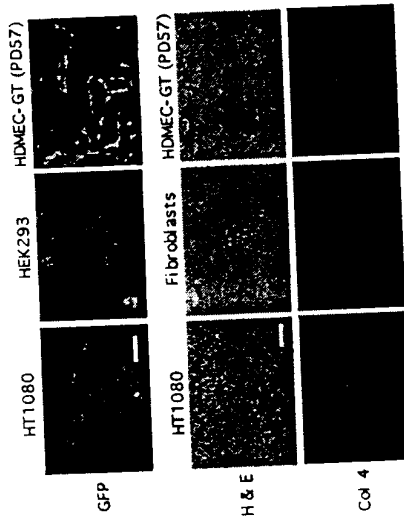


FIGURE 32

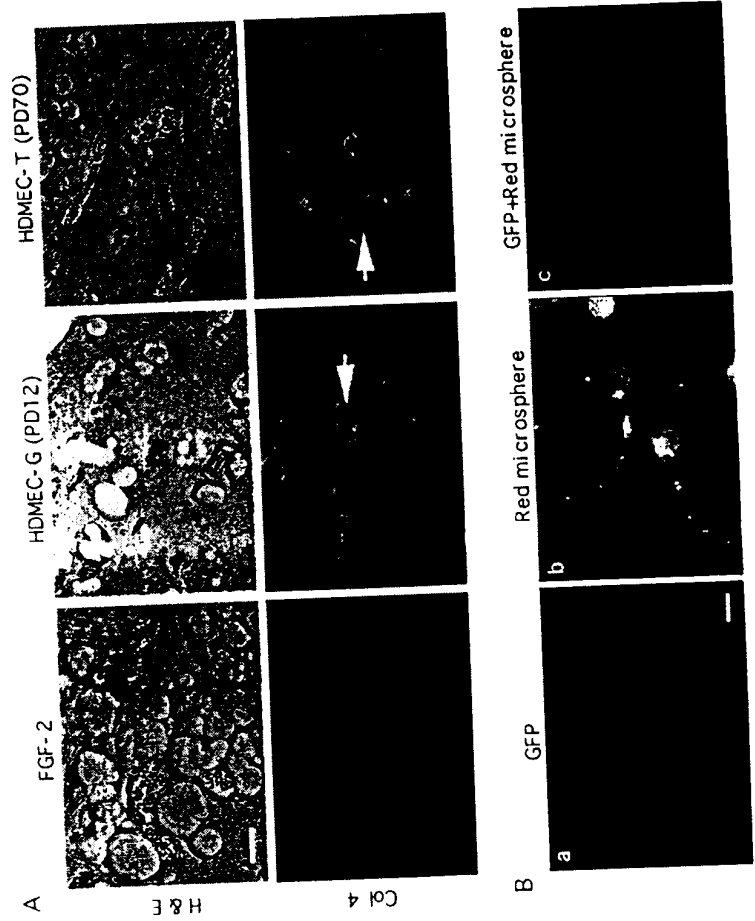


FIGURE 33

